

# Package ‘multiHiCcompare’

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**Title** Normalize and detect differences between Hi-C datasets when replicates of each experimental condition are available

**Version** 1.27.0

**Description** multiHiCcompare provides functions for joint normalization and difference detection in multiple Hi-C datasets. This extension of the original HiCcompare package now allows for Hi-C experiments with more than 2 groups and multiple samples per group. multiHiCcompare operates on processed Hi-C data in the form of sparse upper triangular matrices. It accepts four column (chromosome, region1, region2, IF) tab-separated text files storing chromatin interaction matrices. multiHiCcompare provides cyclic loess and fast loess (fastlo) methods adapted to jointly normalizing Hi-C data. Additionally, it provides a general linear model (GLM) framework adapting the edgeR package to detect differences in Hi-C data in a distance dependent manner.

**Depends** R (>= 4.0.0)

**Imports** data.table, dplyr, HiCcompare, edgeR, BiocParallel, qqman, pheatmap, methods, GenomicRanges, graphics, stats, utils, pbapply, GenomeInfoDbData, GenomeInfoDb, aggregation

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---

cyclic\_loess*Cyclic Loess normalization for Hi-C data*

---

**Description**

Cyclic Loess normalization for Hi-C data

**Usage**

```
cyclic_loess(  
  hicexp,  
  iterations = 3,  
  span = NA,  
  parallel = FALSE,  
  verbose = FALSE  
)
```

**Arguments**

|            |                                                                                                                                            |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp     | A hicexp object                                                                                                                            |
| iterations | The number of iterations (cycles) of loess normalization to perform. Defaults to 3.                                                        |
| span       | The span for loess normalization. Defaults to NA indicating that span will be automatically calculated using generalized cross validation. |
| parallel   | Logical. Should parallel processing be used?                                                                                               |
| verbose    | Logical. Should messages about loess normalization be printed to the screen.                                                               |

**Details**

This function performs cyclic loess normalization on a Hi-C experiment. multiHiCcompare's cyclic loess procedure is a modified version of Ballman's (2004) cyclic loess and the joint loess normalization used in the original HiCcompare. For each unique pair of samples in the hicexp object an MD plot is generated. A loess curve is fit to the MD plot and then the fitted values are used to adjust the data. This is performed on all unique pairs and then repeated until convergence.

**Value**

A hicexp object that has been normalized.

**Examples**

```
#' data("hicexp2")  
hicexp2 <- cyclic_loess(hicexp2, span = 0.7)
```

exportJuicebox

*Export multiHiCcompare results for visualization in Juicebox***Description**

Export multiHiCcompare results for visualization in Juicebox

**Usage**

```
exportJuicebox(
  hicexp,
  logfc_cutoff = 1,
  logcpm_cutoff = 1,
  p.adj_cutoff = 0.01,
  D_cutoff = 1,
  file_name = "juiceboxAnnotations.txt",
  color = "0,0,255"
)
```

**Arguments**

|               |                                                                                                                                                                                        |
|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp        | A hicexp object which has been compared.                                                                                                                                               |
| logfc_cutoff  | The logFC value you wish to filter by. Defaults to 1.                                                                                                                                  |
| logcpm_cutoff | The logCPM cutoff you wish to filter by. Defaults to 1.                                                                                                                                |
| p.adj_cutoff  | The adjusted p-value cutoff you wish to filter by. Defaults to 0.01.                                                                                                                   |
| D_cutoff      | The distance cutoff you wish to filter by. Interactions with a $D < D\_cutoff$ will be filtered. Defaults to 1.                                                                        |
| file_name     | The file name of the text file to be saved.                                                                                                                                            |
| color         | A decimal RGB color code. Should be a character value in form of "0,0,255". Defaults to color code for blue. This will determine the color of the annotations on the Juicebox heatmap. |

**Details**

This function is meant to filter the results of multiHiCcompare and export the significant differentially interacting regions into a text file which can be imported into Juicebox as a 2D annotations file. This will allow you to visualize where your DIRs occur on the heatmap of the interactions. Please see the included vignette on using Juicebox to visualize multiHiCcompare results. This can be accessed with `browseVignettes("multiHiCcompare")`.

**Value**

A text file containing annotations for input into Juicebox.

**Examples**

```
data('hicexp_diff')
exportJuicebox(hicexp_diff, file_name = "juiceboxAnnotations.txt")
```

---

`fastlo`*Perform fast loess normalization on a Hi-C experiment*

---

## Description

Perform fast loess normalization on a Hi-C experiment

## Usage

```
fastlo(  
  hicexp,  
  iterations = 3,  
  span = 0.7,  
  parallel = FALSE,  
  verbose = FALSE,  
  max.pool = 0.7  
)
```

## Arguments

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>hicexp</code>     | A hicexp object                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <code>iterations</code> | The number of iterations (cycles) for fastlo to proceed through.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <code>span</code>       | The span of loess fitting. Defaults to 0.7. To automatically calculate a span using the GCV set <code>span = NA</code> . However note that setting <code>span = NA</code> will significantly slow down the normalization process.                                                                                                                                                                                                                                                                                                                            |
| <code>parallel</code>   | Logical. Should parallel processing be used?                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <code>verbose</code>    | Logical, should messages about the normalization be printed?                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <code>max.pool</code>   | The proportion of unit distances after which all further distances will be pooled. Distances before this value will be progressively pooled and any distances after this value will be combined into a single pool. Defaults to 0.7. Warning: do not adjust this value from the default unless you are getting errors related to the <code>lfproc</code> function or due to sparsity in fastlo normalization. If these errors occur it is due to either sparsity or low variance and <code>max.pool</code> will need to be lowered; typically to 0.5 or 0.6. |

## Details

This function performs the fast loess (`fastlo`) normalization procedure on a `hicexp` object. the fast linear loess ("fastlo") method of Ballman (2004) that is adapted to Hi-C data on a per-distance basis. To perform "fastlo" on Hi-C data we first split the data into `p` pooled matrices. The "progressive pooling" is used to split up the Hi-C matrix by unit distance. Fastlo is then performed on the MA plots for each distance pool. See Stansfield et al (2018) for full description.

## Value

A `hicexp` object that is normalized.

## Examples

```
data("hicexp2")  
hicexp2 <- fastlo(hicexp2)
```

---

`HCT116_r1`*A 4 column sparse matrix for a Hi-C matrix.*

---

**Description**

A matrix object with 4 columns and 56603 rows. Sample 1 of 7 included as example Hi-C data. This is replicate 1 of HCT-116 cell line at 100KB resolution from chHCT116\_r22.

**Usage**`HCT116_r1`**Format**

An object of class `data.frame` with 56603 rows and 4 columns.

**Value**

A matrix

**Source**

Data from Rao 2017. See their website at <http://www.aidenlab.org/> Or the the GEO link to download the data <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104888>

---

`HCT116_r2`*A 4 column sparse matrix for a Hi-C matrix.*

---

**Description**

A matrix object with 4 columns and 57010 rows. Sample 2 of 7 included as example Hi-C data. This is replicate 2 of HCT-116 cell line at 100KB resolution from chHCT116\_r22.

**Usage**`HCT116_r2`**Format**

An object of class `data.frame` with 57010 rows and 4 columns.

**Value**

A matrix

**Source**

Data from Rao 2017. See their website at <http://www.aidenlab.org/> Or the the GEO link to download the data <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104888>

---

HCT116\_r3*A 4 column sparse matrix for a Hi-C matrix.*

---

**Description**

A matrix object with 4 columns and 56744-C data. Sample 3 of 7 included as example Hi-C data. This is replicate 3 of HCT-116 cell line at 100KB resolution from chHCT116\_r22.

**Usage**

```
HCT116_r3
```

**Format**

An object of class `data.frame` with 56744 rows and 4 columns.

**Value**

A matrix

**Source**

Data from Rao 2017. See their website at <http://www.aidenlab.org/> Or the the GEO link to download the data <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104888>

---

HCT116\_r4*A 4 column sparse matrix for a Hi-C matrix.*

---

**Description**

A matrix object with 4 columns and 54307 rows. Sample 4 of 7 included as example Hi-C data. This is replicate 4 of HCT-116 cell line at 100KB resolution from chHCT116\_r22.

**Usage**

```
HCT116_r4
```

**Format**

An object of class `data.frame` with 54307 rows and 4 columns.

**Value**

A matrix

**Source**

Data from Rao 2017. See their website at <http://www.aidenlab.org/> Or the the GEO link to download the data <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104888>

---

`HCT116_r5`*A 4 column sparse matrix for a Hi-C matrix.*

---

**Description**

A matrix object with 4 columns and 55092 rows. Sample 5 of 7 included as example Hi-C data. This is replicate 5 of HCT-116 cell line at 100KB resolution from chHCT116\_r22.

**Usage**`HCT116_r5`**Format**

An object of class `data.frame` with 55092 rows and 4 columns.

**Value**

A matrix

**Source**

Data from Rao 2017. See their website at <http://www.aidenlab.org/> Or the the GEO link to download the data <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104888>

---

`HCT116_r6`*A 4 column sparse matrix for a Hi-C matrix.*

---

**Description**

A matrix object with 4 columns and 55581 rows. Sample 6 of 7 included as example Hi-C data. This is replicate 6 of HCT-116 cell line at 100KB resolution from chHCT116\_r22.

**Usage**`HCT116_r6`**Format**

An object of class `data.frame` with 55581 rows and 4 columns.

**Value**

A matrix

**Source**

Data from Rao 2017. See their website at <http://www.aidenlab.org/> Or the the GEO link to download the data <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104888>

---

|           |                                                                                |
|-----------|--------------------------------------------------------------------------------|
| hg19_cyto | <i>A GenomicRanges object containing centromeric, gvar, and stalk regions.</i> |
|-----------|--------------------------------------------------------------------------------|

---

**Description**

A GRanges object with 2 metadata columns and 70 rows. These ranges indicate the locations of centromeres, stalks, and gvar regions from hg19. Use this for filtering out these regions from your data.

**Usage**

```
hg19_cyto
```

**Format**

An object of class GRanges of length 70.

**Value**

A GRanges object

**Source**

Data from UCSC <http://hgdownload.cse.ucsc.edu/goldenPath/hg19/database/cytoBand.txt.gz>

---

|           |                                                                                |
|-----------|--------------------------------------------------------------------------------|
| hg38_cyto | <i>A GenomicRanges object containing centromeric, gvar, and stalk regions.</i> |
|-----------|--------------------------------------------------------------------------------|

---

**Description**

A GRanges object with 2 metadata columns and 70 rows. These ranges indicate the locations of centromeres, stalks, and gvar regions from hg38. Use this for filtering out these regions from your data.

**Usage**

```
hg38_cyto
```

**Format**

An object of class GRanges of length 70.

**Value**

A GRanges object

**Source**

Data from UCSC <http://hgdownload.cse.ucsc.edu/goldenPath/hg38/database/cytoBand.txt.gz>

---

|              |                                               |
|--------------|-----------------------------------------------|
| Hicexp-class | <i>An S4 class for working with Hi-C data</i> |
|--------------|-----------------------------------------------|

---

**Description**

An S4 class for working with Hi-C data

**Value**

Hicexp class

**Slots**

hic\_table A data.table containing the sparse upper triangular matrix for your Hi-C data.  
 comparison The results of a multiHiCcompare comparison.  
 metadata Data.frame for covariate information.  
 resolution The resolution of the dataset.  
 normalized Indicator for if data has been normalized.

**Examples**

```
data('hicexp2')
hicexp2
```

---

|         |                                                      |
|---------|------------------------------------------------------|
| hicexp2 | <i>hicexp object with 4 samples from two groups.</i> |
|---------|------------------------------------------------------|

---

**Description**

A hicexp object with a hic\_table slot containing 666 rows from chromosome 22 at 1MB resolution.

**Usage**

```
hicexp2
```

**Format**

An object of class Hicexp of length 1.

**Value**

A hicexp object

**Source**

Data from Rao 2017. See their website at <http://www.aidenlab.org/> Or the the GEO link to download the data <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104888>

---

|             |                                                      |
|-------------|------------------------------------------------------|
| hicexp_diff | <i>hicexp object with 7 samples from two groups.</i> |
|-------------|------------------------------------------------------|

---

**Description**

A hicexp object with a hic\_table slot containing 666 rows and a metadata slot containing 3 covariates. Same data as from "hicexp2" object but has been normalized and tested for differences with the hic\_exactTest.

**Usage**

```
hicexp_diff
```

**Format**

An object of class Hicexp of length 1.

**Value**

A hicexp object

**Source**

Data from Rao 2017. See their website at <http://www.aidenlab.org/> Or the the GEO link to download the data <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104888>

---

|               |                                                                           |
|---------------|---------------------------------------------------------------------------|
| hic_exactTest | <i>Perform exact test based difference detection on a Hi-C experiment</i> |
|---------------|---------------------------------------------------------------------------|

---

**Description**

Perform exact test based difference detection on a Hi-C experiment

**Usage**

```
hic_exactTest(hicexp, parallel = FALSE, p.method = "fdr", max.pool = 0.7)
```

**Arguments**

|          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp   | A hicexp object.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| parallel | Logical, should parallel processing be used?                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| p.method | Charact string to be input into p.adjust() as the method for multiple testing correction. Defaults to "fdr".                                                                                                                                                                                                                                                                                                                                                                                                                       |
| max.pool | The proportion of unit distances after which all further distances will be pooled. Distances before this value will be progressively pooled and any distances after this value will be combined into a single pool. Defaults to 0.7. Warning: do not adjust this value from the default unless you are getting errors related to the lfproc function or due to sparsity in fastlo normalization. If these errors occur it is due to either sparsity or low variance and max.pool will need to be lowered; typically to 0.5 or 0.6. |

## Details

This function performs the edgeR exact test on a per distance basis for Hi-C data. It tests for differences between two groups when the groups are the only variable of interest. This is an application of the negative binomial exact test proposed by Robinson and Smyth (2008) for a difference in mean between the groups. These exact tests are applied to the Hi-C data on a distance group basis using "progressive pooling" of distances.

## Value

A hicexp object with the comparison slot filled.

## Examples

```
## Not run:
data("hicexp_diff")
hicexp_diff <- hic_exactTest(hicexp_diff)
## End(Not run)
```

---

|            |                                               |
|------------|-----------------------------------------------|
| hic_filter | <i>Perform filtering on a Hi-C experiment</i> |
|------------|-----------------------------------------------|

---

## Description

Perform filtering on a Hi-C experiment

## Usage

```
hic_filter(hicexp, zero.p = 0.8, A.min = 5, remove.regions = hg19_cyto)
```

## Arguments

|                |                                                                                                                                                                                                                                                                                                             |
|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp         | A hicexp object.                                                                                                                                                                                                                                                                                            |
| zero.p         | The proportion of zeros in a row to filter by. If the proportion of zeros in a row is $\leq$ zero.p the row will be filtered out, i.e. zero.p = 1 means nothing is filtered based on zeros and zero.p = 0 will filter rows that have any zeros.                                                             |
| A.min          | The minimum average expression value (row mean) for an interaction pair. If the interaction pair has an average expression value less than A.min the row will be filtered out.                                                                                                                              |
| remove.regions | A GenomicRanges object indicating specific regions to be filtered out. By default this is the hg19 centromeric, gvar, and stalk regions. Also included in the package is hg38_cyto. If your data is not hg19 you will need to substitute this file. To choose not to filter any regions set regions = NULL. |

## Details

This function is used to filter out the interactions that have low average IFs or large numbers of 0 IF values. If you have already performed filtering when making your hicexp object do not use this again. As these interactions are not very interesting and are commonly false positives during difference detection it is better to remove them from the dataset. Additionally, filtering will help speed up the run time of multiHiCcompare. Filtering can be performed before or after normalization, however the best computational speed gain will occur when filtering is done before normalization.

**Value**

A hicexp object.

**Examples**

```
data("hicexp2")
hicexp2 <- hic_filter(hicexp2)
```

---

hic\_glm

*Function to perform GLM differential analysis on Hi-C experiment*

---

**Description**

Function to perform GLM differential analysis on Hi-C experiment

**Usage**

```
hic_glm(
  hicexp,
  design,
  contrast = NA,
  coef = NA,
  method = "QLFTest",
  M = 1,
  p.method = "fdr",
  parallel = FALSE,
  max.pool = 0.7
)
```

**Arguments**

|          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp   | A hicexp object,                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| design   | A design matrix for the GLM.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| contrast | Numeric vector or matrix specifying one or more contrasts of the linear model coefficients to be tested equal to zero.                                                                                                                                                                                                                                                                                                                                                                                                             |
| coef     | integer or character index vector indicating which coefficients of the linear model are to be tested equal to zero.                                                                                                                                                                                                                                                                                                                                                                                                                |
| method   | The test method to be performed. Should be one of "QLFTest", "LRTest", or "Treat".                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| M        | The log2 fold change value for a TREAT analysis.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| p.method | p-value adjustment method to be used. Defaults to "fdr". See ?p.adjust for other adjustment options.                                                                                                                                                                                                                                                                                                                                                                                                                               |
| parallel | Logical, Should parallel processing be used?                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| max.pool | The proportion of unit distances after which all further distances will be pooled. Distances before this value will be progressively pooled and any distances after this value will be combined into a single pool. Defaults to 0.7. Warning: do not adjust this value from the default unless you are getting errors related to the lfproc function or due to sparsity in fastlo normalization. If these errors occur it is due to either sparsity or low variance and max.pool will need to be lowered; typically to 0.5 or 0.6. |

## Details

This function performs the specified edgeR GLM based test on a per distance basis on the Hi-C data. Distances groups are pooled using "progressive pooling". There are 3 options for the type of GLM based test to be used which is specified with the method option.

QLFTest will use edgeR's glmQLFit and glmQLFTest functions which makes use of quasi-likelihood methods described in Lund et al (2012).

LRTest uses edgeR's glmFit and glmLRT functions which uses a interaction-wise negative binomial general linear model. This method uses a likelihood ratio test for the coefficients specified in the model.

Treat uses edgeR's glmTreat function which performs a test for differential expression with a minimum required fold-change threshold imposed. It tests whether the absolute value of the log2 fold change is greater than the value specified as the M option.

## Value

A hicexp object with a filled in comparison slot.

## Examples

```
## Not run:
data("hicexp_diff")
d <- model.matrix(~factor(meta(hicexp_diff)$group) + factor(c(1,2,1,2)))
hicexp_diff <- hic_glm(hicexp_diff, design = d, coef = 2)
## End(Not run)
```

---

hic\_scale

*Perform library scaling on a hicexp object*

---

## Description

Perform library scaling on a hicexp object

## Usage

```
hic_scale(hicexp)
```

## Arguments

hicexp            A hicexp object.

## Details

This function will perform library scaling on a hicexp object. Scaling is performed separately for each chromosome. This is an alternative normalization method to the cyclic loess and fastlo methods also provided in multiHiCcompare. Use this normalization method if for some reason you do not want to remove trends in the data and only want to normalize based on library size.

## Value

A hicexp object.

**Examples**

```
data("hicexp2")
hicexp2 <- hic_scale(hicexp2)
```

---

|           |                            |
|-----------|----------------------------|
| hic_table | <i>Print the hic_table</i> |
|-----------|----------------------------|

---

**Description**

Print the hic\_table

**Usage**

```
hic_table(x)

## S4 method for signature 'Hicexp'
hic_table(x)
```

**Arguments**

x                      The Hicexp object

**Value**

Hicexp class information

**Examples**

```
data('hicexp2')
hic_table(hicexp2)
```

---

|             |                                              |
|-------------|----------------------------------------------|
| make_hicexp | <i>Make Hi-C experiment object from data</i> |
|-------------|----------------------------------------------|

---

**Description**

Make Hi-C experiment object from data

**Usage**

```
make_hicexp(
  ...,
  data_list = NA,
  groups,
  covariates = NULL,
  remove_zeros = FALSE,
  zero.p = 0.8,
  A.min = 5,
  filter = TRUE,
  remove.regions = hg19_cyto
)
```

**Arguments**

|                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ...            | Hi-C data. Data must in sparse upper triangular format with 4 columns: chr, region1, region2, IF or in 7 column BEDPE format with columns chr, start1, end1, chr, start2, end2, IF.                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| data_list      | Alternate way to enter data. If you have your Hi-C data in the form of a list already with each entry of the list representing a sample use this option.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| groups         | A vector of the experimental groups corresponding to each Hi-C data object entered. If it is not in factor form when entered it will be converted to a factor.                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| covariates     | Optional data.frame containing covariate information for your Hi-C experiment. Some examples are enzyme used, batch number, etc. Should have the same number of rows as the number of Hi-C data objects entered and columns corresponding to covariates.                                                                                                                                                                                                                                                                                                                                                                                |
| remove_zeros   | Logical, should rows with 1 or more zero IF values be removed?                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| zero.p         | The proportion of zeros in a row to filter by. If the proportion of zeros in a row is $\leq$ zero.p the row will be filtered out, i.e. zero.p = 1 means nothing is filtered based on zeros and zero.p = 0 will filter rows that have any zeros.                                                                                                                                                                                                                                                                                                                                                                                         |
| A.min          | The minimum average expression value (row mean) for an interaction pair. If the interaction pair has an average expression value less than A.min the row will be filtered out.                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| filter         | Logical, should filtering be performed? Defaults to TRUE. If TRUE it will filter out the interactions that have low average IFs or large numbers of 0 IF values. As these interactions are not very interesting and are commonly false positives during difference detection it is better to remove them from the dataset. Additionally, filtering will help speed up the run time of multiHiCcompare. Filtering can be performed before or after normalization, however the best computational speed gain will occur when filtering is done before normalization. Filtering parameters are controlled by the zero.p and A.min options. |
| remove.regions | A GenomicRanges object indicating specific regions to be filtered out. By default this is the hg19 centromeric, gvar, and stalk regions. Also included in the package is hg38_cyto. If your data is not hg19 you will need to substitute this file. To choose not to filter any regions set regions = NULL. NOTE: if you set filter = FALSE these regions will NOT be removed. This occurs in conjunction with the filtering step.                                                                                                                                                                                                      |

**Details**

Use this function to create a hicexp object for analysis in multiHiCcompare. Filtering can also be performed in this step if the filter option is set to TRUE. Filtering parameters are controlled by the zero.p and A.min options.

**Value**

A hicexp object.

**Examples**

```
# load data in sparse upper triangular format
data("HCT116_r1", "HCT116_r2", "HCT116_r3", "HCT116_r4",
      "HCT116_r5", "HCT116_r6")
# make groups & covariate input
groups <- factor(c(1, 1, 1, 2, 2, 2))
```

```

covariates <- data.frame(enzyme = factor(c('mobi', 'mboi', 'mboi',
      'dpnii', 'dpnii', 'dpnii')), batch = c(1, 2, 1, 2, 1, 2))
# make the hicexp object
hicexp <- make_hicexp(HCT116_r1, HCT116_r2, HCT116_r3, HCT116_r4,
      HCT116_r5, HCT116_r6, groups = groups,
      covariates = covariates)

```

manhattan\_hicexp

*Manhattan plot function for results of multiHiCcompare*

## Description

Manhattan plot function for results of multiHiCcompare

## Usage

```

manhattan_hicexp(
  hicexp,
  pval_aggregate = "standard",
  return_df = FALSE,
  p.adj_cutoff = 0.05,
  plot.chr = NA
)

```

## Arguments

|                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp         | A hicexp object that has had differences detected                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| pval_aggregate | string denoting the p-value method to use for plotting. Options are "standard", "fisher", "lancaster", "sidak", and "count". "standard" plots a manhattan plot using all individual p-values (very slow, use with caution). "fisher", "lancaster", or "sidak" methods use the Fisher's, Lancaster, or the Sidak method, respectively, for combining p-values for each region which are then plotted on the $-\log_{10}(\text{p-value})$ Y-axis. "count" summarizes the number of times a region was detected as significant (see "p.adj_cutoff" parameter), plotted on Y-axis. The higher the dots are, the more significant/more frequent a region was detected as significantly differentially interacting. See <code>?topDirs</code> |
| return_df      | Logical, should the data.frame used to generate the plot be returned?                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| p.adj_cutoff   | The adjusted p-value cutoff to be used for calling an interaction significant. This is only used if method = 'count'. Defaults to 0.05.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| plot.chr       | A numeric value indicating a specific chromosome number to subset the plot to. Defaults to NA indicating that all chromosomes will be plotted.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

## Details

This function is used to create a manhattan plot for the significance of all genomic regions in the dataset. These correspond to the rows (or columns) of the upper triangle of the full Hi-C matrix. Each genomic region of the Hi-C dataset has multiple interactions it is involved in and the significance of all of these can be visualized with `pval_aggregate = "standard"`. Alternatively the p-values for all these interactions can be combined using either Fisher's, or the Lancaster or the Sidak method of combining p-values. Additionally the "count" option will plot based on the number of times each region was found to be involved in a significantly different interaction. The manhattan plot can be used to identify "hotspot" regions of the genome where major differences seem to be located based on the results of a multiHiCcompare analysis.

**Value**

A manhattan plot and optionally the data.frame used to generate the manhattan plot.

**Examples**

```
data("hicexp_diff")
manhattan_hicexp(hicexp_diff, pval_aggregate = "fisher")
```

---

|              |                                                                  |
|--------------|------------------------------------------------------------------|
| MD_composite | <i>Plot a composite MD plot with the results of a comparison</i> |
|--------------|------------------------------------------------------------------|

---

**Description**

Plot a composite MD plot with the results of a comparison

**Usage**

```
MD_composite(hicexp, plot.chr = NA, D.range = 1)
```

**Arguments**

|          |                                                                                                                                                                                                                                                                                          |
|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp   | A hicexp object which has had a multiHiCcompare comparison step performed on it.                                                                                                                                                                                                         |
| plot.chr | A specific chromosome or set of chromosome which you want to plot. This should be a numeric value, i.e. to plot chromosome 1 set plot.chr = 1, to plot chromosomes 1 and 5 set plot.chr = c(1, 5). Defaults to NA indicating that all chromosomes present in the hicexp will be plotted. |
| D.range  | Allows for subsetting of the plot by Distance. Set to proportion of total distance that you want to be displayed. Value of 1 indicates that the entire distance range will be displayed. Defaults to 1.                                                                                  |

**Value**

An MD plot

**Examples**

```
data("hicexp_diff")
MD_composite(hicexp_diff)
```

---

|           |                                                          |
|-----------|----------------------------------------------------------|
| MD_hicexp | <i>Make MD plots for all combinations of a condition</i> |
|-----------|----------------------------------------------------------|

---

**Description**

Make MD plots for all combinations of a condition

**Usage**

```
MD_hicexp(hicexp, prow = 3, pcol = 3, plot.chr = NA, plot.loess = FALSE)
```

**Arguments**

|            |                                                                                                                                                                                                                                                                                          |
|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp     | A hicexp object.                                                                                                                                                                                                                                                                         |
| prow       | The number of rows to use for the grid of MD plots. Defaults to 3.                                                                                                                                                                                                                       |
| pcol       | The number of columns to use for the grid of MD plots. Defaults to 3.                                                                                                                                                                                                                    |
| plot.chr   | A specific chromosome or set of chromosome which you want to plot. This should be a numeric value, i.e. to plot chromosome 1 set plot.chr = 1, to plot chromosomes 1 and 5 set plot.chr = c(1, 5). Defaults to NA indicating that all chromosomes present in the hicexp will be plotted. |
| plot.loess | Logical, should a loess curve be plotted over the MD plots. Note setting this to TRUE will increase the computational time for plotting.                                                                                                                                                 |

**Value**

A set of MD plots.

**Examples**

```
data("hicexp2")
MD_hicexp(hicexp2)
```

---

|      |                           |
|------|---------------------------|
| meta | <i>Print the metadata</i> |
|------|---------------------------|

---

**Description**

Print the metadata

**Usage**

```
meta(x)

## S4 method for signature 'Hicexp'
meta(x)
```

**Arguments**

|   |                   |
|---|-------------------|
| x | The Hicexp object |
|---|-------------------|

**Value**

Hicexp class information

**Examples**

```
data('hicexp2')
meta(hicexp2)
```

---

|            |                                                          |
|------------|----------------------------------------------------------|
| normalized | <i>Print the indicator for if the data is normalized</i> |
|------------|----------------------------------------------------------|

---

**Description**

Print the indicator for if the data is normalized

**Usage**

```
normalized(x)

## S4 method for signature 'Hicexp'
normalized(x)
```

**Arguments**

x                      The Hicexp object

**Value**

Hicexp class information

**Examples**

```
data('hicexp2')
normalized(hicexp2)
```

---

|           |                                                                                                                  |
|-----------|------------------------------------------------------------------------------------------------------------------|
| perm_test | <i>Perform a permutation test to check enrichment of a genomic feature with DIRs detected by multiHiCcompare</i> |
|-----------|------------------------------------------------------------------------------------------------------------------|

---

**Description**

Perform a permutation test to check enrichment of a genomic feature with DIRs detected by multi-HiCcompare

**Usage**

```
perm_test(
  hicexp,
  feature,
  p.adj_cutoff = 10^-10,
  logfc_cutoff = 1,
  num.perm = 1000,
  pval_aggregate = "max"
)
```

**Arguments**

|                |                                                                                                                                                                                                                                                                                                                                                                                                               |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp         | A Hicexp object which has been compared.                                                                                                                                                                                                                                                                                                                                                                      |
| feature        | A GRanges object containing locations for a genomic feature you would like to test for enrichment in the differentially interacting regions (DIRs).                                                                                                                                                                                                                                                           |
| p.adj_cutoff   | The adjusted p-value cutoff for declaring a region significant. See ?topDirs for more information. Defaults to 10^-10                                                                                                                                                                                                                                                                                         |
| logfc_cutoff   | The log fold change cutoff for a region to be declared significant. See ?topDirs for more information. Defaults to 1.                                                                                                                                                                                                                                                                                         |
| num.perm       | The number of permutations to run. Defaults to 1000.                                                                                                                                                                                                                                                                                                                                                          |
| pval_aggregate | Method to aggregate region-specific p-values. If a region differentially interacts with several other regions, the p-values are aggregated using a 'max' method (Default, select maximum p-value, most conservative), or the Fisher ('fisher'), Lancaster ('lancaster'), or Sidak ('sidak') methods (see 'aggregate' package). regions, it is assigned a single p-value aggregated from several. See ?topDirs |

**Value**

The permutation p-value

**Examples**

```
## Not run:
data("hicexp_diff")
data("hg19_cyto")
perm_test(hicexp_diff, hg19_cyto)

## End(Not run)
```

---

plot\_counts

---

*Plot the count results from topDirs*


---

**Description**

Plot the count results from topDirs

**Usage**

```
plot_counts(dirs, plot.chr = NA)
```

**Arguments**

|                       |                                                                                                                                                |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>dirs</code>     | The output of the <code>topDirs</code> function when the <code>return_df</code> option is set to "bed".                                        |
| <code>plot.chr</code> | A numeric value indicating a specific chromosome number to subset the plot to. Defaults to NA indicating that all chromosomes will be plotted. |

**Value**

A plot.

**Examples**

```
data('hicexp_diff')
dirs <- topDirs(hicexp_diff, return_df = 'bed')
plot_counts(dirs)
```

---

|                         |                                              |
|-------------------------|----------------------------------------------|
| <code>plot_pvals</code> | <i>Plot the p-value results from topDirs</i> |
|-------------------------|----------------------------------------------|

---

**Description**

Plot the p-value results from `topDirs`

**Usage**

```
plot_pvals(dirs, plot.chr = NA)
```

**Arguments**

|                       |                                                                                                                                                |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>dirs</code>     | The output of the <code>topDirs</code> function when the <code>return_df</code> option is set to "bed".                                        |
| <code>plot.chr</code> | A numeric value indicating a specific chromosome number to subset the plot to. Defaults to NA indicating that all chromosomes will be plotted. |

**Value**

A plot.

**Examples**

```
data('hicexp_diff')
dirs <- topDirs(hicexp_diff, return_df = 'bed')
plot_pvals(dirs)
```

---

|              |                                                                    |
|--------------|--------------------------------------------------------------------|
| pval_heatmap | <i>Function to visualize p-values from multiHiCcompare results</i> |
|--------------|--------------------------------------------------------------------|

---

**Description**

Function to visualize p-values from multiHiCcompare results

**Usage**

```
pval_heatmap(hicexp, alpha = NA, chr = 0)
```

**Arguments**

|        |                                                                                                                                                                                                                                         |
|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp | A hicexp object that has been normalized and has had differences detected.                                                                                                                                                              |
| alpha  | The alpha level at which you will call a p-value significant. If this is set to a numeric value then any p-values $\geq$ alpha will be set to 1 for the visualization in the heatmap. Defaults to NA for visualization of all p-values. |
| chr    | The numeric value for the chromosome that you want to plot. Set to 0 to plot all chromosomes in the dataset.                                                                                                                            |

**Details**

The goal of this function is to visualize where in the Hi-C matrix the differences are occurring between two experimental conditions. The function will produce a heatmap of the  $-\log_{10}(\text{p-values}) * \text{sign}(\log\text{FC})$  to visualize where the significant differences between the datasets are occurring on the genome.

**Value**

A heatmap

**Examples**

```
data("hicexp_diff")
pval_heatmap(hicexp_diff, chr = 22)
```

---

|            |                             |
|------------|-----------------------------|
| resolution | <i>Print the resolution</i> |
|------------|-----------------------------|

---

**Description**

Print the resolution

**Usage**

```
resolution(x)

## S4 method for signature 'Hicexp'
resolution(x)
```

**Arguments**

x                      The Hicexp object

**Value**

Hicexp class information

**Examples**

```
data('hicexp2')
resolution(hicexp2)
```

---

results

*Print the results*

---

**Description**

Print the results

**Usage**

```
results(x)

## S4 method for signature 'Hicexp'
results(x)
```

**Arguments**

x                      The Hicexp object

**Value**

Hicexp class information

**Examples**

```
data('hicexp2')
results(hicexp2)
```

---

|                    |                                                |
|--------------------|------------------------------------------------|
| show,Hicexp-method | <i>Print information about a HiCexp object</i> |
|--------------------|------------------------------------------------|

---

**Description**

Print information about a HiCexp object

**Usage**

```
## S4 method for signature 'Hicexp'  
show(object)
```

**Arguments**

|        |                 |
|--------|-----------------|
| object | A Hicexp object |
|--------|-----------------|

**Value**

HiCexp information

---

|            |                                                                 |
|------------|-----------------------------------------------------------------|
| smartApply | <i>Function to apply either biocParallel or standard lapply</i> |
|------------|-----------------------------------------------------------------|

---

**Description**

Function to apply either biocParallel or standard lapply

**Usage**

```
smartApply(parallel, x, FUN, ...)
```

**Arguments**

|          |                                                             |
|----------|-------------------------------------------------------------|
| parallel | Logical, should parallel processing be used?                |
| x        | The main list object which the function will be applied to. |
| FUN      | The function to be applied.                                 |
| ...      | Additional arguments for bplapply or lapply.                |

**Value**

results of lapply or bplapply

topDirs

*Filter results of multiHiCcompare***Description**

Filter results of multiHiCcompare

**Usage**

```
topDirs(
  hicexp,
  logfc_cutoff = 1,
  logcpm_cutoff = 1,
  p.adj_cutoff = 0.01,
  D_cutoff = 1,
  return_df = "pairedbed",
  pval_aggregate = "max"
)
```

**Arguments**

|                |                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hicexp         | A hicexp object which has been compared.                                                                                                                                                                                                                                                                                                                                                        |
| logfc_cutoff   | The logFC value you wish to filter by. Defaults to 1.                                                                                                                                                                                                                                                                                                                                           |
| logcpm_cutoff  | The logCPM cutoff you wish to filter by. Defaults to 1.                                                                                                                                                                                                                                                                                                                                         |
| p.adj_cutoff   | The adjusted p-value cutoff you wish to filter by. Defaults to 0.01.                                                                                                                                                                                                                                                                                                                            |
| D_cutoff       | The distance cutoff you wish to filter by. Interactions with a $D < D\_cutoff$ will be filtered. Defaults to 1.                                                                                                                                                                                                                                                                                 |
| return_df      | The format for the data.frame returned by the function. Options are "bed" and "pairedbed" (Default).                                                                                                                                                                                                                                                                                            |
| pval_aggregate | Method to aggregate region-specific p-values. If a region differentially interacts with several other regions, the p-values are aggregated using a 'max' method (Default, select maximum p-value, most conservative), or the Fisher ('fisher'), Lancaster ('lancaster'), or Sidak ('sidak') methods (see 'aggregate' package). regions, it is assigned a single p-value aggregated from several |

**Details**

This function is meant to filter the results of multiHiCcompare. The top differentially interacting regions (DIRs) can be returned by using this function. When the return\_df = "bed" option is set the resulting data.frame can be input into the plot\_pvals or plot\_counts functions to visualize the top DIRs.

**Value**

A data.table containing the filtered results.

**Examples**

```
data('hicexp_diff')
topDirs(hicexp_diff)
```

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